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LIMNOLOGICAL SAMPLE AND DATA COLLECTION METHODS USED BY THE MUSKOKA LAKES AQUATIC ASSESSMENT UNIT

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LIMNOLOGICAL SAMPLE AND DATA COLLECTION METHODS USED BY THE MUSKOKA LAKES AQUATIC ASSESSMENT UNIT

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Introduction

There have been a number of sample collection protocols used for both limnological and biological sample collection in the Muskoka Lakes since 1986. Many of these methods were developed and used by the Ontario Ministry of the Environment and Energy (MOEE) field staff at the Dorset Research Centre (DRC) who conducted limnological investigations in the Lakes between 1986 and 1989.

In 1993, a co-operative program between the Ministry of Natural Resources (OMNR), Muskoka Lakes Fisheries Assessment Unit (MLFAU) and the MOEE, Lake Assessment Group (DRC) was initiated to continue the collection of limnological, biological and fisheries information on the Muskoka Lakes. The combined group is now called the Muskoka Lakes Aquatic Assessment Unit (MLAAU). In the first year of the initiative, water quality data were collected as they had been since 1986 using the DRC protocols for stratified, volume-weighted samples. In addition, simple euphotic zone composite samples were collected simultaneously at all locations on each visit. It was felt that for most applications, euphotic zone composites would provide sufficient information and avoid the complexities involved with volume-weighted sample collection. The intention was to develop simple lake assessment techniques that could be used elsewhere without the need for complex sampling gear and convoluted collection protocols.

Volume-weighted sampling was discontinued after the 1993 field season in favour of composite sampling methods. Other biological collection techniques, such as those used for the collection of zooplankton samples, were unchanged.

This manual outlines the current methods used by the MLAAU for the collection of biological (limnology related) samples including zooplankton and benthos. Standardized methods developed elsewhere, primarily by the Long Range Transport of Atmospheric Pollutants group (LRTAP), and used here for the collection of benthos are also summarized. Other widely used, standard limnological sample collection methods are not outlined in this report if they have been replaced by current sample collection methods.

This report does not outline any methods used to collect fisheries related data.

Transparency

Secchi Depth

Secchi disc depths are determined at each visit to any sampling location. The Secchi depth ('SD', Fig. 1) is determined as the depth mid-way between where the disc first disappears on descent and then reappears with recovery (Hutchinson, 1957) and is recorded to the nearest 0.1 m. Care should be taken to keep the observer's face reasonably close to the water and to observe the disc at a location where the surface of the water is shaded (Fig.1). Observations should be made as close to mid-day as is practical (Hutchinson, 1957). Recent additions to the protocol caution against the use of polarizing glasses. There have been discrepancies noted between the Secchi depth determined on the same day by OMNR and MOEE staff (Fig. 2). This has been tentatively attributed to problems associated with the high sides on the MOEE boat but bias is also often evident between observers. There is no effective way, however, to eliminate many of the subjective aspects of Secchi depth determinations.

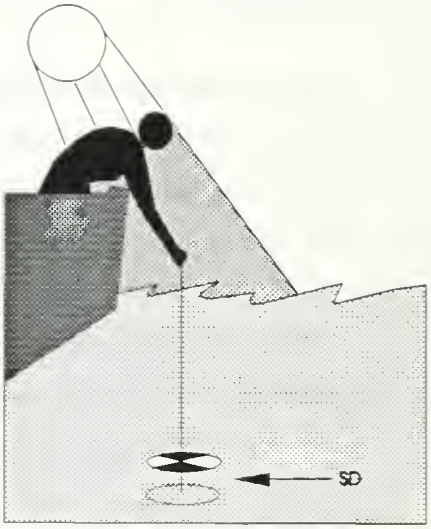


Fig. 1. Use of the Secchi disc to determine transparency.

Secchi depths are doubled to determine the depth of the euphotic zone. This depth is used to collect *euphotic zone composite* samples (see water chemistry) for water chemistry and chlorophyll analysis.

Secchi disc observations are routinely collected as a simple and inexpensive way to gather long-term comparative data that reflects the trophic status of a water body. Secchi disc observations have also been shown to be a sensitive indicator of long term changes in trophic status. Smeltzer (1989) found that Secchi disc measurements were less subject to temporal variability than either chlorophyll or total phosphorous (TP) measurements and, as such, could provide a better

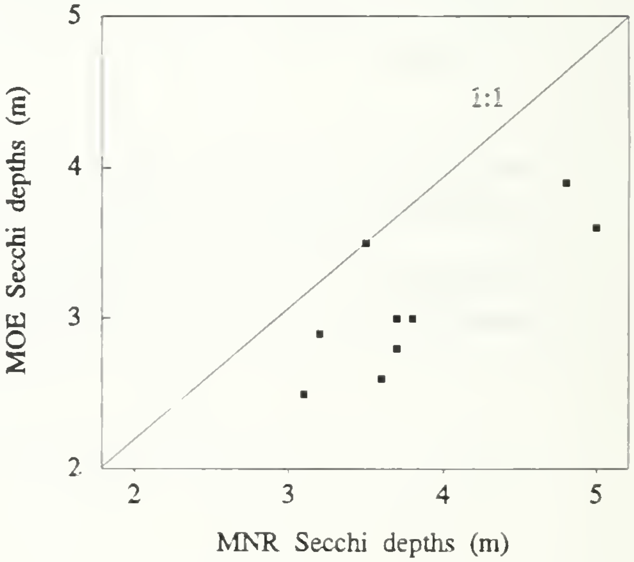


Fig. 2. Comparison between Secchi depths observed by OMNR ar
MOEE staff.

monitoring tool for early detection of eutrophication. Transparency observations, however, may be influenced by factors other than those related to trophic status (most commonly Dissolved Organic Carbon) and should therefore be interpreted together with TP and/or chlorophyll data. Marshall (1994) recently reported improvements in the measured usefulness of trophic status indicators based on Secchi, chlorophyll and TP measurements when these indicators where corrected for water colour and aquatic plant biomass.

The observation of long-term trends in transparency should be based on ice-free-mean Secchi depths. These values should be derived from (minimally) monthly observations (May to Nov) but the number of observations required each ice-free season will increase with higher trophic status. Twice monthly observations will usually give results that are within 10% of the mean (95% confidence) (Clark and Hutchinson 1992). Generally, field staff should collect Secchi data for all visits to any location.

Long term averages can be determined to within 20% with a single year of data and within 10% with 2-5 years of data. Care must be taken to ensure that there is not a trend in the long-term data before quoting long-term averages.

At the DRC, Secchi depth data is secured electronically to the ORACLE data base through data fields on submission sheets and does not require transfer from field note books. Other field staff or individuals who perform Secchi depth determinations as part of various separate projects should ensure that the data will make it somehow 'out' of the field books. There tends to always be a great deal more data of this type in existence than appears in the records.

Researchers who plan to collect transparency data for the first time can manufacture their own Secchi discs. Any material that sinks can be cut into an 8 inch disc and painted with alternating black and white quadrants. Steel plate and plexiglass have both been successfully used to manufacture Secchi discs. The attached ropes used to measure the actual depths should be the subject of some concern. They should be checked and calibrated frequently since most materials change length through stretching, or by wetting. It is worthwhile to use a brand of non stretching rope to precisely lower any sampling apparatus to a distinct depth.

Sampling Zones and Layers

Some thought must be given to the portion or zone of the water body that the water quality samples must represent. There are several commonly sampled zones and varying methods for combining the water from each zone into distinct samples. The most common zones and sampling methods are outlined below.

Whole lake - Whole lake samples are usually taken in the spring or fall when the lake is thermally mixed. In these cases, the sample is presumed to represent the entire water body. These samples are collected as composites of the water column from the surface to a predetermined depth or to the bottom. This is often 0 - 5 m or 0 - 1 m from the bottom. Staff at MLAAU collect euphotic zone composites all year long. When these are collected at times when the lake is mixed they are coded as whole lake composites. Similarly, sampling programs which collect volume-weighted samples should consider volume-weighted samples that are collected at turnover to be equal to whole-lake samples.

Euphotic Zone - The euphotic zone is defined as the layer of water between the surface and a depth equal to twice the Secchi depth. This zone is commonly sampled to represent the layer of water that supports the majority of plankton growth within the lake.

Epilimnetic - Epilimnion samples are most often collected along with metalimnion and hypolimnion samples to describe conditions within the individual zones of a stratified lake. They can usually be collected as 5 m composites without regard to the actual depth of the epilimnion. More care should be taken to match composite depths to actual epilimnetic depths if it is suspected that the epilimnion is either very shallow or very deep. The premise remains, though, that this is a mixed layer. Epilimnetic samples are often pooled to derive stratified season averages.

Metalimnetic - Metalimnetic samples are most often collected along with epilimnion and hypolimnion samples to describe conditions within the mixed and unmixed layers of a stratified lake. Temperature data is required to define the upper and lower limits of the layer.

Hypolimnetic - It is difficult to collect representative samples from the hypolimnion since the layer is generally not mixed. This can be overcome by collecting composite samples, by volume-weighting samples or by describing the most extreme conditions by collecting samples from 1 m off the bottom. For parameters such as oxygen, individual observations can be collected at intervals throughout the hypolimnion to obtain 'profile' data.

One meter off bottom - See hypolimnetic samples

Composite Sampling Methods - There are several commonly used methods to collect composite samples. The composite bottle has been used for many years and simply involves lowering and raising a weighted bottle through the desired depths. Large diameter hoses can be lowered into the water column, and their contents trapped and retrieved as a composite. Peristaltic pumps are commonly used to combine water from distinct depths. Apparatus normally associated with grab sampling such as Van Dorn or Kemmerer bottles can be triggered at numerous depths and samples then pooled to provide composites over a given range. Volume-weighting as described below is a form of composite sampling.

Volume Weighting methods - It is often desirable to combine water from different strata in proportions equal to the actual volumes of lake water in those strata. This is usually required for mass balance applications. Peristaltic pumps or Van Dorn bottles are used to retrieve the correct proportions of water from different depths which are usually then combined as zone composites ie: hypolimnetic (volume-weighted) composites.

Grab Samples - Grab samples are used to describe the water quality conditions at a specific points in the lake rather than for a zone. They are usually collected with Van Dorn or Kemmerer bottles but can be collected with pumps or by simply filling bottles at the surface.

Sampling Seasons - The results from individual samples are often pooled to derive seasonal means. Common seasons include the ice-free season which will include all samples collected in open water, the stratified season which includes all samples collected while the lake is stratified and the overturn season which includes samples from either spring or fall overturn.

Sample Collection Methods

All recent MLAAU water samples for chemical analysis are collected as 'euphotic zone composites'. The decision to collect euphotic zone composites considers the fact that the euphotic zone represents the layer of the lake which is responsible for generating algal growth. The euphotic zone is defined as the layer of water bounded by the surface and the depth at which there is approximately 1% of the surface light. It is commonly approximated by simply doubling the Secchi depth. Generally, the use of a composite bottle to collect these samples is recommended. There seems to be no evidence that more complex sample collection methods will give results that are different or superior to those obtained with a simple composite bottle for the collection of either epilimnetic or euphotic zone water. Samples collected for chlorophyll analysis by both composite bottle and volume weighting at distinct depths have shown the same results (see Fig. 5). This can be considered a reasonably rigorous test since chlorophyll is one of the few parameters that could show some stratification even in mixed layers.

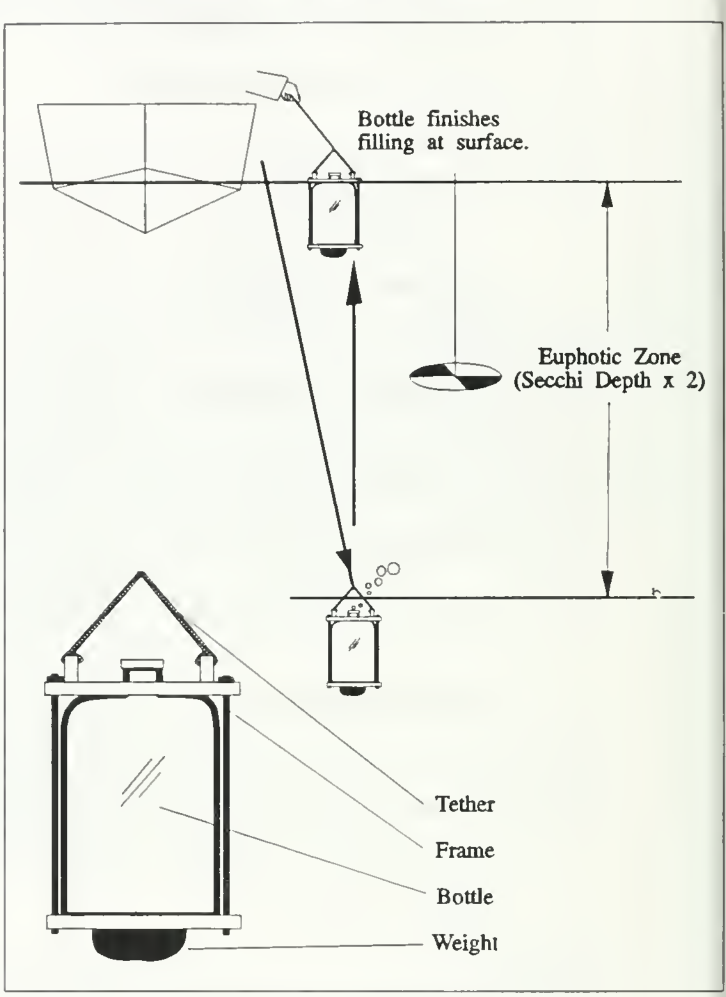

Bottle finishes filling at surface.
Euphotic Zone (Secchi Depth x 2)
Tether
Frame
Bottle
Weight

Fig. 3. Composite sampling apparatus and techniques.

Samples are collected by lowering a weighted composite bottle to the desired depth (z = Secchi depth x 2) and then retrieving it to the surface at a rate that will allow the bottle to just finish filling upon return to the surface (Fig. 3). This allows the collection of samples that are subject to very minimal 'handling and container effects', which is important from a quality control and sample contamination standpoint. Some caution is required with respect to the size of the composite bottle aperture. It should be of a size sufficient to let the bottle fill throughout the entire descent and ascent. Actual aperture sizes will be determined in relation to bottle volume and are best determined through trial end error. Bottles can be lowered more slowly if the aperture is small but bottles with wide openings may need to have the inlet partially blocked (made smaller) to reduce fill rates to accommodate the sampling of very deep euphotic zones.

It is important to note that composite bottles will provide samples that do not contain equal proportions of water from all depths since sinking and retrieval rates as well as fill rates are not constant. For example, weighted bottles will sink slowly as they first begin to fill and consequently they will spend more time filling in the upper depths. This is offset by the fact that they will fill faster due to the pressure at greater depths but at the same time sink faster as they fill and become less buoyant (therefore spending less time passing through the greater depths). The impact of these complications is lessened by the fact that the euphotic zone is usually composed mostly of epilimnetic water which is theoretically mixed. This will lessen the impact of collecting unequal volumes from different depths. In cases where the bottom depth of the euphotic zone is less than or equal to the depth of the epilimnion (which is often the case) the entire sample will be taken within the mixed layer. If portions of the metalimnion are included in a euphotic zone composite there may be some measurable variation if different proportions of each layer are combined but this does not seem to be the case for most parameters in dilute water lakes. Comparisons between samples collected to observe various parameters from all layers including the euphotic zone at 36 stations in the three Muskoka Lakes is presented in detail in the MLAAU, *State of the Lakes Progress Report* (1996 in prep). Chlorophyll samples collected in 1993 at locations where the euphotic zone contained portions of the metalimnion by both composite bottle and peristaltic pump volume-weighting methods yielded identical results. This was seen as a test of the 'worst case' situation since algae (and hence chlorophyll) have been demonstrated through fluorometry to occasionally stratify dramatically, especially in the vicinity of the metalimnion (Clark, unpublished data). This situation would be expected to produce greater variation between different types of composite samples in more eutrophic lakes but the differences between concentrations of chlorophyll at various depths may not be that extreme in more dilute lakes.

Occasionally, in situations where hypolimnetic water quality is a concern, additional samples are collected at 1 m from the bottom using a Kemmerer or Van Dorn bottle. Hypolimnetic water chemistry may show a great degree of seasonal variation for many parameters, especially in more enriched lakes or where hypolimnetic anoxia occurs. These include, notably; phosphorus, nitrogen, and iron and oxygen but may include any parameter on a lake specific basis. It is therefore important to have a parameter specific and distinct need for any data that is collected.

Water Sampling Schedules

Water sampling for chemical characterization is preferably done at spring or fall turnover when the lakes are 'mixed'. In this way, general water chemistry sampling for an entire year can be completed with a single visit to the lake. These samples are subject to some year-to-year variation but this can usually be observed within a few years for most parameters. The data obtained from a single visit when the lake is 'mixed' will be more valuable than the pooled data from several visits if those sampling dates represent times when distinct samples will not represent the whole lake. For example, a single spring-overturn phosphorus determination can be used as input to trophic status models but samples from June, July and August will represent only a portion of an ice-free mean and, as such, represent no single term that is useful as input to the model. It is important to note that smaller lakes with fetch < 1.4 km, (Molot et al. 1992) may not completely mix in the spring and this should be considered if the lake is to be characterized using the data from a single visit.

It is often difficult to typify a lake for some parameters with a single visit if those parameters show a high degree of within-year or between-year variation. Examples would be chemical parameters observed in the hypolimnion of anoxic lakes, or any tests such as chlorophyll that involve living plants which display seasonal growth patterns. Attempts to typify a lake for parameters such as chlorophyll usually cannot be based on a single value, although there may be some merit to using the results from a single visit if the sample coincides with peak summer values in lakes that do not display seasonal blooms, Marshall, 1995. Specific research designed to observe these seasonal patterns often require frequent sampling intervals that may include time periods when lakes are stratified. For chlorophyll, euphotic zone composites should always be collected. The values for most other parameters collected as euphotic zone composites will closely match epilimnetic composites or epilimnetic volume-weighted samples. Protocols used to sample distinct layers (epilimnion, metalimnion, hypolimnion) in lakes during stratification are described elsewhere (MLAAU 1993, internal methods manual).

The number of years of data that is required to observe long-term means is parameter specific since each test will display individual variance. These data for the trophic status parameters are shown in Table 2 (from Clark and Hutchinson 1992). The use of a single number for complex analysis or for input to models should therefore consider between-year or seasonal variability. It is, however, common to accept the characterization of a water body based on the results from the most recent visit without concern for the year-to-year variance associated with the individual parameters.

Table 2: Trophic status parameter sample frequencies and protocols.

			Samples/year		**Number of years**	
	Derivation	**Sample method**	10% of mean	20% of mean	10% of mean	20% of mean*
TP(so)	usually a single sample	whole lake composite	1	1	10	2
TP(if)	average of all samples collected for ice free period	composites when lake is mixed volume weighted during stratification	9-13 (bi-weekly)	4-5 (monthly)	5	1
TP(epi)	average of all samples collected during stratification	epilimnetic composite	19	5	7	2
Chl a (ss)	average of all samples collected during stratification ie: self help programs	euphotic zone composites	less than for Chl(if) should use Chl(if) if spring/fall blooms expected			
Chl a (if)	average of all samples collected for ice free period	euphotic zone composites	10	5	>5	2-5
Oxygen	usually profile data	at distinct depths by oxygen meter	sample frequency based on final use of data			
Secchi	observation	Secchi disc	11-17 (weekly)	3-4 (monthly)	2-5	1

** 95 % confidence*
(so = spring overturn, if = ice free season, epi = epilimnetic average, ss = stratified season)

Sample locations

Smaller lakes require measurements at only one mid-lake station (the deepest location) while larger lakes or lakes with localized influences may require the establishment of several sampling locations. Researchers should base sample locations on the specific intended use for the data.

Water Quality Parameters

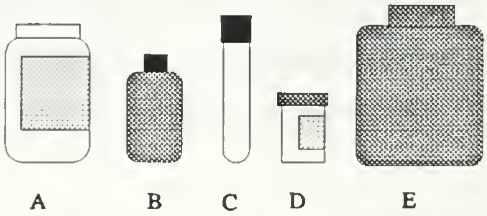
Each research or monitoring related use for a database may require the collection of different parameters. This can promote a desire to collect as much information as possible. It is, however, costly to use large parameter groups and it can be wasteful if the data are never put to use. It is also risky to increase sample frequency without consideration for the seasonal variation in the parameters that are observed. For example, a single total phosphorus measurement at spring turnover has great value for characterizing the trophic status of the lake or as input to trophic status models. Even several similar measurements during the stratified season will not have equal value since they will represent only portions of the ice-free-epilimnetic total phosphorus concentration. Many samples are required to derive such a number accurately. For these reasons, the parameters observed should be program specific and address the desire to answer specific questions as much as possible.

General characterization parameters collected by the MOEE which can be used as a guideline for establishing general water quality include: *pH, alkalinity, total Kjeldahl nitrogen, nitrate, ammonia, iron, conductivity, colour, dissolved inorganic and organic carbon, sulphate, chlorophyll a and total phosphorus.* Secondary parameters collected less often, usually once per year at spring overturn, include: *aluminum, fluoride, manganese, chloride, potassium, magnesium, calcium, silica and sodium.* These may be omitted entirely unless specific needs for the data can be identified.

Sample Containers

Sample container and submission protocols vary with each parameter and should be verified through contact with MOEE labs or by contacting MOEE field staff at the MLAAU. Most of the above tests are submitted in 500 ml PET jars. Exceptions to this are outlined in Figure 4. As a precaution, all bottles and caps should be rinsed once with the sample prior to filling. Bottles with gas caps are filled to contain no air and all others are filled close to the brim. All samples should be kept cool while en route to the lab.

Fig. 4. Sample containers: A = 500ml PET jar, B = 200ml opaque, polyethylene gas tight bottle for pH, alk, C = 35 ml borosilicate tube with teflon lined cap for total phosphorus and gas cap for DIC, D = 100ml plastic jar for metals, E = 1 litre opaque, polyethylene bottle for chlorophyll samples.



Oxygen and Temperature

Methods

Oxygen and temperature profiles are recorded in-situ using a digital YSI oxygen/temperature meter. Profiles are observed at 1, or 2 m intervals depending on the degree of detail required to observe epi-meta and meta-hypo boundaries. Generally most observations are in two meter intervals. For very deep and oxygenated hypolimnia where concentrations do not change over wide depth ranges, 4 m intervals are sufficient. MNR field crews determine oxygen and temperatures at one meter intervals and should continue to do so to maintain database consistency.

Operation of oxygen/temperature meters is straightforward as outlined in the meter-owner's manual. Several universal cautions should, however, be observed. Oxygen/temperature probes should be left at the desired depth long enough to equilibrate. This process may take several minutes at very low oxygen concentrations although newer meters seem to come to equilibrium much faster than older models. Probes without stirrers will have to be gently 'jigged' to ensure a movement of water across membrane surfaces. Anoxic conditions can ruin the probe membranes such that the probe should be left for minimal time in anoxic water. This is important if more stations are to be sampled later in the day since replacing membranes in the field will make meter calibration more difficult (see below). It is not necessary to verify anoxic conditions between where they are first encountered and the bottom since anoxic water will not overlay more oxygenated water. The probe will still have to be lowered through anoxic layers if temperature data is required but this can be done more quickly if only the temperature data is recorded. Values are entered in a waterproof field book and transferred manually to the data base.

Meter Calibration

Oxygen meter membranes are changed approximately monthly but would probably last much longer. The need to change membranes for different models of oxygen meter will be evident by difficulties encountered with daily calibration procedures. Membranes should be changed when meters cannot hold their calibration. Meter calibration for most models is by air saturation and this was shown on three occasions to give results to within 0.1 ppm of the lab meter at the DRC which is calibrated using saturated water at a constant temperature. Several checks by MLAUU staff using Winkler determinations showed close agreement between samples and in-situ measurements. It was felt that the handling of Winkler samples presented more potential for error than the chance that the meter would be incorrect. The field and lab meter never disagreed by more than 0.1ppm in situations where the variables were strictly controlled. Care should be taken to complete the air calibration at a time when the temperature of the sensor is constant. This is best done in the lab prior to each use when the meter and probes have been at room temperatures overnight. The need to attain constant temperatures for calibration is the main reason to avoid membrane changes in the field. The calibration on most days did not need to be adjusted but the need for adjustment was seen as evidence that the membrane required changing. Some calibration is required after changing the membrane.

Oxygen / Temperature Profile Sampling Schedules

In the past, oxygen profile measurements were typically made at the deepest location in the lake on a monthly basis throughout the open water season. These types of data are difficult to interpret because concentrations change spatially and temporally within a given year, and can also show considerable variation between years. One method of addressing this variation is to examine only the end of summer profiles. This eliminates the need to evaluate seasonal changes by concentrating on the 'worst case' profiles at the time of year when oxygen concentrations in the hypolimnion are at the open water minimum. It is preferable, when classifying lakes in this manner, to use the long-term average profile derived from several years of data which will counteract the effects of inter-annual variation. Molot and Dillon (1991) recommend the collection of 6 years of data to derive long-term average profiles. This approach will allow managers to describe the average condition in the hypolimnion at the end of summer (September 01 +/- 2 wks) and compare between-lake differences under similar conditions. This approach to oxygen monitoring suggests that field crews concentrate on the collection of end-of-summer profiles specifically between August 15 and September 15 (Molot. et al. 1992). Temperature profiles should also be collected to determine hypolimnetic boundaries. Core data bases could benefit more from the collection of profiles from several different lakes circa the first of September than from a series of monthly observations from the same lake over the course of the summer.

Temperature profiles may be required at more frequent intervals for specific research purposes. It may be necessary to monitor seasonal changes in the boundaries of stratified layers in the lake or to verify turnover in the spring or fall.

Chlorophyll

Chlorophyll samples have been collected historically by a number of methods. Most of these are intended to be a composite of the euphotic zone (Secchi depth x 2). Chlorophyll samples collected in the past using Dorset volume-weighting protocols were actually volume-weighted-euphotic-zone composites. If the euphotic zone is mixed, the nature of these samples should not matter but it has been shown through fluorometry profiles that the euphotic zone is often stratified with respect to chlorophyll concentrations. This could potentially cause problems with representing the volume-weighted samples as 'composites' since distinct volumes of water are pumped from exact depths and then combined. In 1993, samples were collected using peristaltic pumps (Dorset protocols) and at the same time a second sample was taken using a simple composite bottle. These were shown to give identical results (Fig. 5).

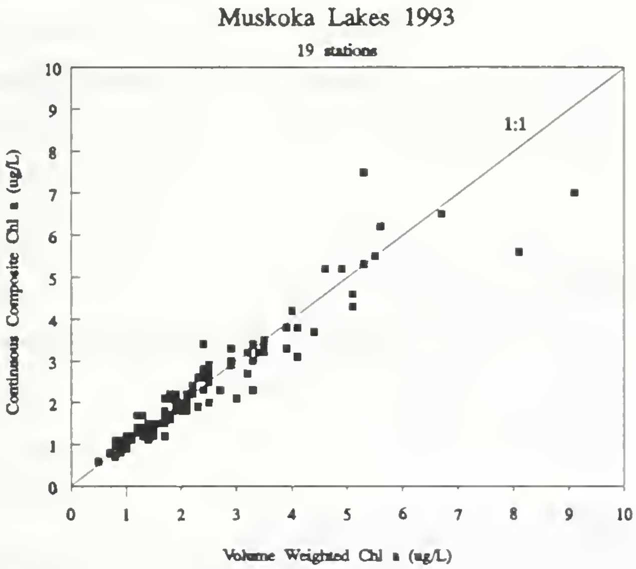


Fig. 5. Comparison of composite bottle and volume weighted euphotic zone composite, chlorophyll data.

Chlorophyll is currently sampled by filling a 1 litre, opaque, plastic container from the euphotic zone composite bottle (which is used for the collection of all other water chemistry parameters). Samples should be kept cool for transport to the lab and fixed with 0.5 ml/ L saturated magnesium carbonate within 8 hours of collection.

Chlorophyll Sampling Schedules

Chlorophyll is often collected as an indicator of trophic status primarily because algal growth is the most evident result of a change in the trophic status of the lake. Chlorophyll, however, tends to show a great deal of seasonal and inter-annual variation especially in the more eutrophic systems. Since these seasonal patterns cannot be represented by a single or even several chlorophyll observations it is often necessary to collect numerous samples throughout the year to determine meaningful 'ice free average' concentrations. It is, on average, necessary to collect more than 10 samples per season to derive averages which are within 20% of the mean (95% confidence) and 30 to 50 samples to be within 10% (Clark and Hutchinson, 1992). Establishing a long term mean (data from

Dorset lakes), will require 1 to 4 years of data to be within 20% and 3 to 16 years for 10%. Generally, the more eutrophic the system, the more years of data that will be required. In addition, chlorophyll samples tend to be perishable and very susceptible to a number of 'handling' problems between the time of sampling and sample analysis. While there may be merit in quoting individual chlorophyll concentrations to quantify the extent of an algal bloom or to indicate point-in-time concentrations, it is both costly and labour intensive to use chlorophyll as a tool to reflect trophic status.

Phytoplankton Enumeration

Samples for phytoplankton enumeration are collected as euphotic zone composites. They are poured into 500 ml PET jars and fixed (in the field) with 1 ml Lugols fixative stain*. Samples do not require refrigeration prior to delivery to the lab.

* Recipe for Lugols fixative stain.

5 gm iodine
10 gm potassium iodide
100 ml distilled water

To save enumeration costs it is often practical to combine portions of each monthly sample at a given station into a single 'seasonal composite enumeration sample' which will describe the general or relative numbers of organisms present during the entire ice-free season. Sample combination is performed by the enumeration staff at the MOEE, Rexdale Lab. Collection strategies currently used by MLAAU staff include the collection of both individual monthly samples for stations of interest and monthly samples which will be combined into single (ice-free composite) samples to provide more general seasonal information. Phytoplankton enumeration samples are usually collected in conjunction with regular zooplankton samples.

Zooplankton

Modified Wisconsin Net Volume-Weighted Composites

Regular zooplankton samples are collected once per month using exact Dorset protocols. These are volume-weighted, 80 micron, modified Wisconsin net ('Dorset Net') hauls (Fig. 6). Volume- weighting is accomplished by combining the material obtained in four separate hauls which have individual haul depths established to yield approximate volume-weighted samples. This is to ensure that proportions in the sample are similar to the proportions of animals in the entire water body rather than just in the water column. The depths of each of the four hauls must be predetermined and this information must accompany the sampler in the field.

The net should be retrieved at a rate of at least one meter per second to stay above meter stall speeds and all four hauls at each site should be metered. One metered but netless haul is conducted to allow calculation of net efficiency. The depth of the netless haul should be the same as the depth of the longest sample haul at each location (to conform with ZEBRA data entry protocols). The net is removed by loosening the hose clamp at the bottom of the meter. The net should be left off for the netless haul at the following site so the net need only be removed once for every two locations.

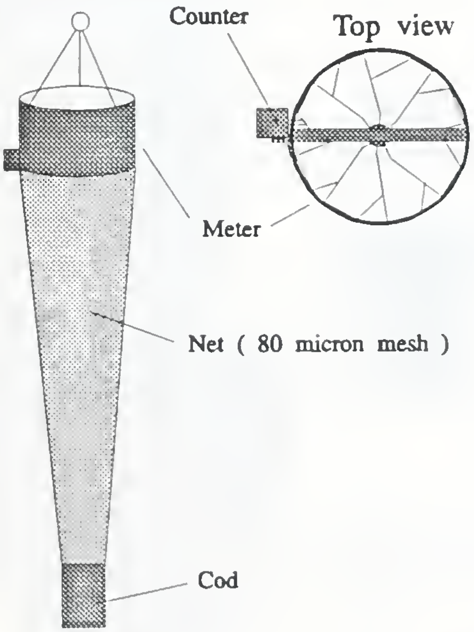


Fig. 6. The Dorset, modified Wisconsin Zooplankton net.

The material from each haul is rinsed from the cod end using lake water and a Nalgene Unitary wash (squirter) bottle into a 500ml PET jar . The material from all four hauls are combined into a single PET jar (Fig. 7). All efforts should be made to reduce the volume of sample which will minimize the use of preservatives and reduce the space required for sample storage. Once the four individual haul samples have been combined into a 500 ml PET jar they can be returned to the cod end and further concentrated by removing a great deal of the water used for the four rinses. The sample will then contain the lake water from only one rinse. If possible these samples should then be transferred into smaller glass jars (approx. 55 ml) to facilitate transport to counting personnel and eventual long-term storage at the Dorset Research Centre. The preferred situation would be for the field samples to be collected in-situ into the smaller glass jars but this is often not possible especially if large numbers of *Holopedium* are present. Zooplankton samples are preserved with equal volumes of 8% buffered sugar formalin* preservative to yield a final sample strength of 4% formalin. Care should be taken to label all fixative containers and fixed samples with the correct MSDS information.

* Recipe for buffered sugar formalin:

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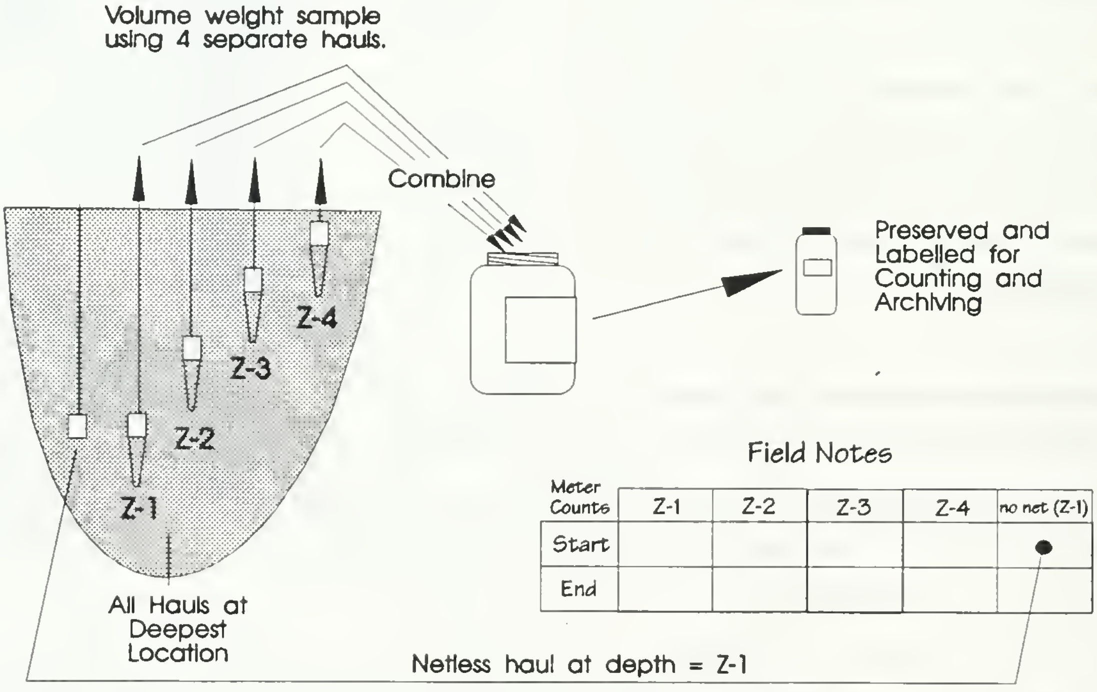
360 g sugar
620 ml formaldehyde
5 L distilled water
43 drops 0.5 N, NaOH.


Volume weight sample using 4 separate hauls.
Combine
Z-4
Z-3
Z-2
Z-1
Preserved and Labelled for Counting and Archiving
All Hauls at Deepest Location
Field Notes
Netless haul at depth = Z-1

Meter Counts	Z-1	Z-2	Z-3	Z-4	no net (Z-1)
Start					●
End					

Fig.7. Schematic of methods used to volume weight Zooplankton Hauls.

Bythotrephes Biology Sample Collection

Additional zooplankton samples are collected with a large mesh (250µ), large diameter (0.75m) plankton net (Fig. 8) to determine biology related information for the recent invading zooplanktor *Bythotrephes cederstroemi* or 'spiny water flea'. Each haul was metered using a detachable, Rigoshi, plankton net, flow meter to determine net efficiencies. There are no stall speeds determined for the large net but there is evidence that it must be retrieved as quickly as possible to ensure proper meter operation. This, at best, would be around 0.5 m/sec but this speed will be difficult to maintain over greater haul depths. It also appears that these faster haul speeds will create a bow wave effect that may reduce the real efficiency of the net. It is difficult to verify, but the optimal haul speed for capturing zooplanktors may be below the speed required for proper operation of the meter. This is a result of improper mating of nets and meters. Average efficiencies measured at IM2 where haul depths are 18m were around 85% whereas those measured at IM5 where depths are 40 m were around 75%. This infers that the net cannot be hauled by hand fast enough to avoid Rigoshi meter stall speeds or possibly that stall speeds can only be exceeded over shorter haul distances. This does not, however validly compare real net efficiencies at different haul speeds. In separate patchiness studies where numerous timed hauls were taken at several locations, the number of animals captured was not related to measured haul speeds or depths, which indicates extreme patchiness. In these tests, the observed efficiencies are similar to those observed for the Wisconsin net (80%).

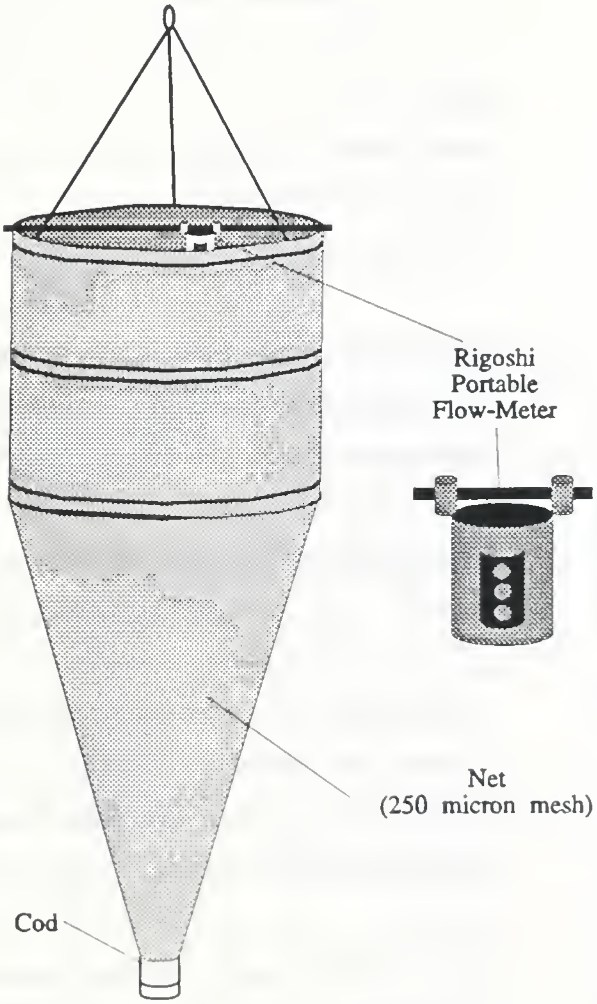


Fig. 8. The 0.75m diameter 250 micron Btyhotrephes net.

Bythotrephes distinguish themselves by a large barbed caudal appendage which tends to easily catch onto net surfaces (Fig. 9). It was found that a complete second rinse of the net would deliver numbers of additional creatures to the cod. There were seldom any remaining after a second rinse such that one rinse was established as routine practice. Care must be taken for similar reasons to ensure that all creatures are washed from the cod. They tend to stick to all mesh sizes but more so to the larger meshed, more brittle fabric and less to smaller mesh or softer fabric.

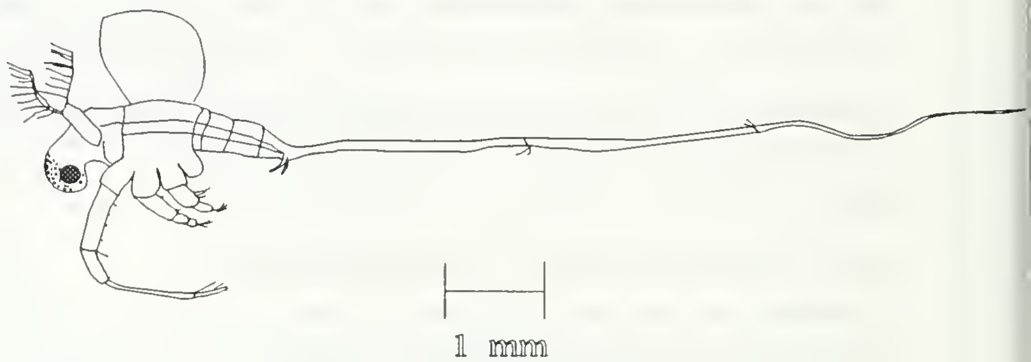


Fig. 9. Schematic of *Bythotrephes cederstroemi*.

This is also reason enough to be extremely careful when using nets in lakes which do not contain *Bythotrephes* to avoid accidental introduction of the creatures into the lake. Nets should be soaked in 2% chlorine or left to dry for extended periods between use if transfer between lakes is intended.

Desiccated *Bythotrephes* in the sample are evidence that the net was not rinsed adequately on a previous sample date.

Initial investigations indicate that *Bythotrephes* is an epi-metalimnetic animal with probably very few individuals inhabiting the hypolimnion. Protocols that are designed to verify presence/absence can therefore be restricted to sampling the top 15 m. More rigorous sampling would be required to verify vertical density distributions and abundance estimates would be extremely difficult to derive due to the patchy distribution of the animals.

Samples for *Bythotrephes* are preserved, as for the Wisconsin hauls, by combining equal volumes of sample and 8% sugar formalin to arrive at a final concentration of 4% formalin. As with the regular zooplankton samples, volumes should be kept to a minimum.

Benthos

Part of the LRTAP biological effects monitoring program includes the survey of benthic organisms. For each lake, four sampling activities are used:

1. Kick and sweep sampling of the shallow (<1m) littoral areas to collect Ephemeroptera, Trichoptera, Odonata, Amphipoda, Gastropoda and other misc. shallow water benthos;
2. Deep water dredge samples to collect Amphipoda, Mollusca and Insecta from sub-littoral areas;
3. Baited wire traps to collect Decapoda and Hirudinea;
4. Night time vertical net tows to capture taxa such as *Mysis* or *Chaoborus* which are semi-benthic during the day but planktonic at night.

1. Kick and sweep collection of littoral benthos

There are a number of reasons to collect quantitative and qualitative data on the benthic community. This type of sampling is conducted at MLAAU specifically to search for long-term changes in the near-shore benthos. The 'kick and sweep' sampling technique is designed to collect the maximum number of species together with minimum bottom debris and with minimum habitat disruption. Exact protocols for kick and sweep sampling of near-shore areas should be developed after closely consulting the guidelines developed for such sampling methods by LRTAP (summarized in Reid et al. 1994) and through consultation with field biologists. Efforts should be made to choose representative sampling areas and attempt to collect the maximum possible number of species present in each area.

Samples should be collected at a time of year when the benthic animals are either about to emerge as adults (the spring) or about to overwinter as mature nymphs (late Fall). Collection timetables should endeavour to capture the maximum number of invertebrates at a stage where taxonomy is easiest, or at least possible. For example, the smallest juveniles of many species are virtually impossible to distinguish.

Sampling strategies that specify a set length of time to kick and sweep will provide information about the 'relative' abundance of creatures as long as specific details about the nature of the kicking and sweeping are controlled. Strategies that regulate exact areas to be sampled will provide information about 'absolute' abundance.

Dorset protocols involve the collection of animals in the late fall by kicking and sweeping along transects that run perpendicular from the shore to a depth of 1 m. Organisms are captured using a 250µ mesh "D" net. The sampler disturbs the bottom by foot and sweeps the disrupted material into the net, usually while walking slowly towards shore (Fig. 10). Sample material is collected into and transported in 20 L, plastic pails. Effort is constrained to a set period of time such that as many transects as possible were sampled in a 10 min period. There have been concerns that this method does not provide information about absolute abundance and may not adequately represent relative abundance unless the kicking and sweeping intensity of the samplers are strictly controlled. However, Reid et al. (1995) have confirmed that this time-limited protocol has high repeatability. The length and number of transects sampled should be recorded to ensure consistency across different types of substrate and different personnel.

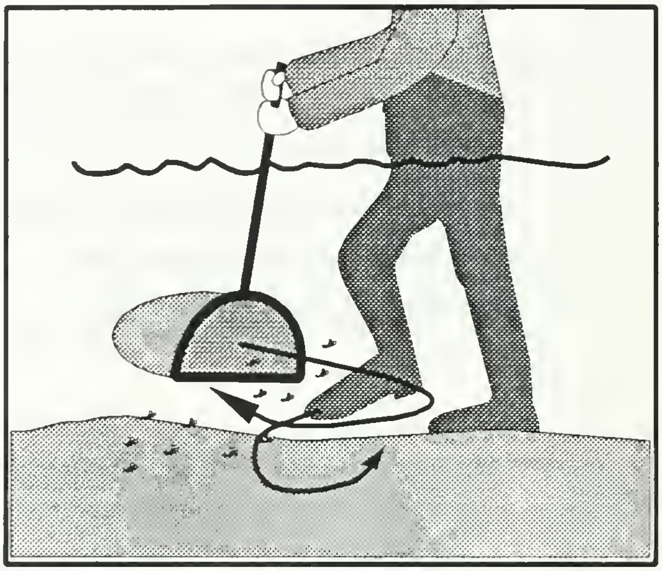


Fig. 10. Schematic of kick and sweep methods.

Samples were initially picked 'live' to remove as many obvious and large organisms as possible. Care should be taken to complete the live picking as soon as possible to keep the organisms from eating one another in the pail. All samples should be kept as cold as possible prior to picking and preserving. Organisms are picked and sorted roughly into 6 classes, each in its own vial preserved with alcohol as follows:

1. **Odonates:** Dragonflies, Damselflies.
2. **Amphipoda:** Scuds, *Hyallela*, *Crangonyx*.
3. **Chironomidae**: Midge larvae, blood worms.
4. **Miscellaneous**: Beetles, mites, blood suckers, etc
5. **Trichoptera**: Caddisflies including empty houses.
6. **Pelecypoda, Mollusca:** Snails, clams, etc.

The residual from the pre-picked samples is then transferred to smaller plastic jars, preserved with ethyl alcohol, stained and left for 3 to 5 days. The material is then rinsed and repicked to remove the smaller organisms. The six vials per sample are then shipped to the contracted taxonomist who identifies and counts the animals.

Rapid Bioassessment using Kick and Sweep Techniques

Researchers may require information about the benthic invertebrates in lakes or streams for a number of reasons. There may be concerns about the effects of changes in water quality on the invertebrate community. They may require an index to compare individual lakes or streams in relation to a larger set of reference communities or the invertebrates may be of interest simply as one component in a broader food web analysis. Unfortunately, removing the animals from the debris in benthic invertebrate samples is usually time consuming and they require substantial funds for the identification of the different taxa. Following this, there is often disagreement surrounding the analysis and interpretation of the data.

There have been many attempts to simplify the collection and interpretation of benthic invertebrate information. Researchers at the DRC have conducted nearshorebenthic invertebrate index studies on a large number of lakes using LRTAP protocols (Shaw et al. 1992). As noted above, these were expensive and exhaustive studies. Recently, attempts have been made to utilize simpler, rapid-bioassessment techniques that will provide general information about the nature of the benthic community without the need for exhaustive sample picking or professional identification of organisms. Current rapid-bioassessment techniques provide information about benthic communities of the littoral zones in a range of lake types that can be evaluated against the more detailed and comprehensive LRTAP data base. Researchers interested in rapid bioassessment of benthic communities could collect comparative data by using standard DRC collection protocols as outlined below.

Samples are collected using kick and sweep methods as described above. Nearshore samples from lakes are collected over a 10 min sample interval and streams over 1 min. In lakes, transects are swept from shore to a depth of 1 m whereas $1m^2$ quadrats are swept in streams. All sample material is transferred to a 20 L pail or other suitable container with a tight fitting lid. This material is then further concentrated by removing the large debris such as leaves, bark, sticks, and stones using a large mesh sieve (50mm). Care must be taken not to discard animals adhering to the large debris. Small material such as silt, sand and detritus is removed next using a 1000µ sieve. The residue should fit into a large jar. These steps are usually completed in the field. The residue is then either preserved and stained or may be immediately picked live. The methods are similar in either case.

Picking and Enumerating Rapid Bioassessment Samples

Randomly selected subsamples of approximately 15 ml of material are placed in a white, porcelain pan and all animals are removed and preserved in alcohol. As they are picked, each animal is enumerated by 'checking' a box in a bench sheet in one of 27 categories. These categories are class, order, sub-order or family depending on the category. Pictures of representative animals for each category are on the bench sheet (Fig.11). This process is continued until 100 animals are counted. The goal is to select 100 animals in roughly the same proportions that they were in the parent sample. Both picked samples, and remaining parent sample are preserved. It may be desirable at some point later to fully pick some samples to verify that picked and parent sample proportions are similar.

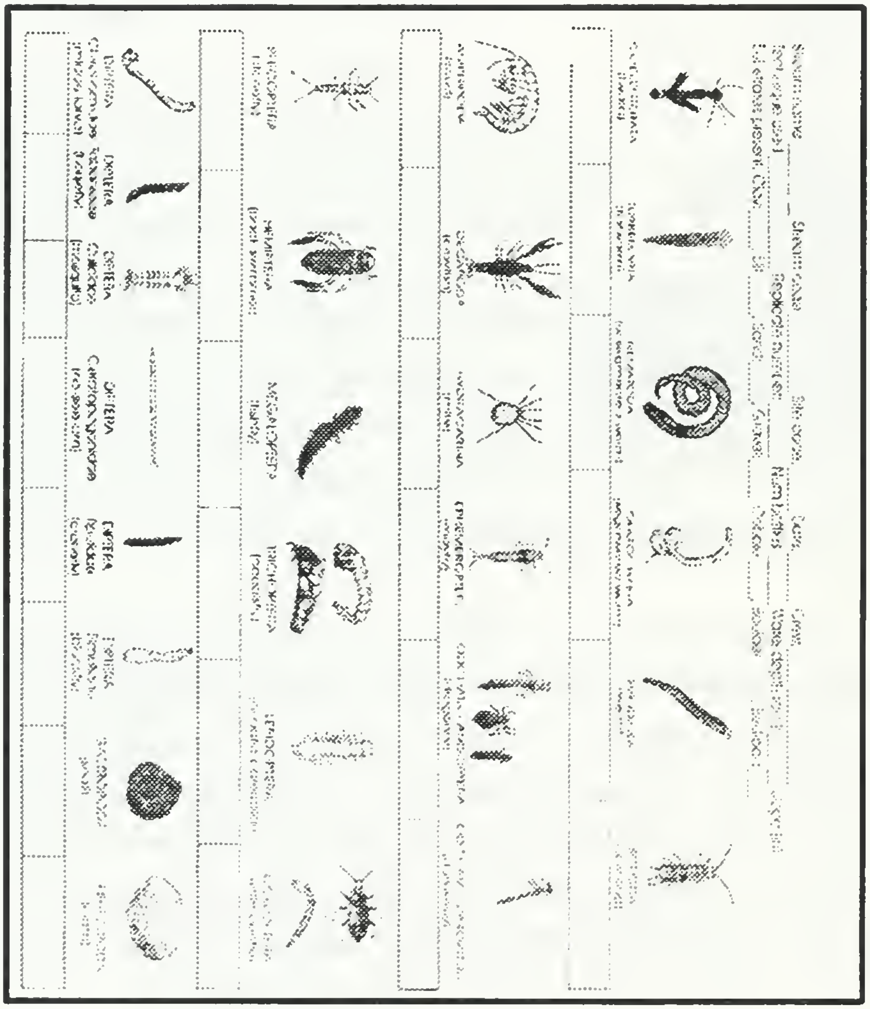


Fig.11. Example of bench sheet used for rapid-bioassessment techniques.

The results of the rapid bioassessment method are summarized by the field bench sheet. For many purposes, there is no need to have the preserved sample professionally identified. The results as they relate to the actual categories and numbers in each category of the 100 animals picked are most easily interpreted when compared to a large number of rapid bioassessment samples from reference areas that span a large range of known conditions, i.e. lake type, water quality, etc. These types of reference sets are currently being established at the DRC (Reid et al. 1994). Alternatively, individual samples can be interpreted by an entomologist for broad characterization of benthic community types. The samples may require identification to species for some animals if more detailed interpretations relating to the sensitivity of the organisms to stressors are warranted. In any event, as more samples re collected by the same protocols, the more useful the individual samples will become since each sample will contribute to the range of possible conditions in the reference database..

In general, rabid bioassessment techniques are used as a screening tool to search for potential problem areas. More intensive methods requiring detailed taxonomic identifications are generally utilized as follow-up techniques once a potential problem ares has been identified

2. Sub littoral collections

Organisms may be collected from the deeper sediment in the lake using an Eckman dredge. Samples are picked and preserved as for the kick and sweep method.

3. Crayfish

The following is excerpted from David et. al. 1994. Protocols generally follow those outlined by the federal-provincial Long Range Transport of Airborne Pollutants (LRTAP) programme (Shaw et al. 1992).

Sampling for relative abundance of crayfish should begin during the last week of June or the first week of July. Each lake will require two days to sample. Modified wire mesh minnow traps with funnel entrances enlarged to 3.5 cm are set on day one and retrieved on day two. Each lake should have three crayfish sampling sites identified and suitably mapped or located so that they can be resampled in subsequent years. These sites should represent the major habitat types characteristic of the lakes in the region, ideally detritus, macrophyte, and rocks. Detailed substrate descriptions should be recorded with standardized classification codes as follows:

Code	Description	Particle size* (mm)	Phi scale
BDR	Bedrock - continuous sheets		
BOU	Boulder	> 256	-8
COB	Cobble	64 - 256	-6,-7
PEB	Pebble	32-64,16-32,8-16,4-8	-5,-4,-3,-2
CSD	Coarse Sand	0.5 - 1	1
MSD	Medium Sand	0.25 - 0.5	2
FSD	Fine Sand	0.125 - 0.25	3
SLT	Silt	.0039 - .0625	5,6,7,8
CLA	Clay	< .0039	9
MUD	Mud		
FMN	Ferromanganese nodules		
LOS	Highly organic flocculent		
GRA	Gravel (Granule-Wentworth)	2 - 4	-1
CPO	Coarse particle organic (leaves, needles, small sticks)		
FPO	Fine particle organic		
LOG	Submerged logs		
MAC	Macrophytes: emergent or submergent		

* Wentworth classification after Cummings (1962)

On both sampling days, the following should be recorded: Lake name, date, cloud cover (as proportion of sky, eg., 0/10), water and air temperature, trap set or retrieval times, plus other relevant information.

On day 1, 18 traps are set at each site. The traps are set in 3 lines perpendicular to the shore from 1 to 6 m deep and 5 m apart. In shallow lakes, lines can be set to 4 m if necessary (record in field book). Each trap is set containing a perforated film canister filled with fish flavoured, canned cat food as bait.

Substrate information should be recorded on day one i.e., 20% logs, 50% cobble, 30% coarse sand. Percent cover of macrophytes should also be entered as a separate number. For each site there should also be a shoreline description i.e., 20% pine, 40% sedges, 40% rock. Any obvious landmarks should also be recorded i.e., 20 m west of white cottage dock etc.

On day 2, site number and time are recorded when collecting traps. Crayfish information is recorded in the following sequence:

Line	Depth	Species	Sex	Carapace Hardness	Carapace Length	Remarks
1	5	Ov	FII	SH	33.6	RCM

Line Trap lines at each site are numbered from left to right when facing the shore

Depth Depths are at the trap location in meters (e.g. trap#1 is usually at 1m depth, #2 at 2m and so on to trap #6 at 6m)

Species Species are recorded by First letter of genus and First letter of species:

Cambarus bartoni	Cb
Cambarus robustus	Cr
Orconectes immunis	Oi
Orconectes obscurus	Oo
Orconectes propinquus	Op
Orconectes rusticus	Or
Orconectes virilus	Ov

Sex The female symbol is used to record female crayfish. Males are recorded according to sexual maturity i.e., Sexually functional (form I) - FI Not sexually functional (form II) - FII

Carapace Hardness Subjective rating assigned by gently squeezing the thorax between the thumb and forefinger:

VS	very soft - recent moult - carapace collapses
S	soft
SH	medium harness, compresses easily
HS	medium harness, compresses with force
H	hard
VH	very hard, dark colour, stained with diatoms

Length the dorsal carapace length is measured with vernier calipers as the distance between the tip of the acumen (the extreme anterior end of the rostrum) and the posterior end of the cephalothorax (in tenths of mm. -e.g., 33.6 mm.)

Remarks as defined by LRTAP

Condition	Crayfish Condition Indicators
DED	Dead specimen
RCR	Right chela regenerated (smaller than left)(DRC code only)
LCR	Left chela regenerated (smaller than right)(DRC code only)
RCM	Right chela missing
LCM	Left chela missing
CBM	Both chela missing
DAN	Damaged animal usually exoskeleton wounds

ACU	Acumen broken - affects carapace length measurement
NWI	newly moulted - very soft carapace
PMI	Pre moult individual
DSC	Dirty or stained carapace
CGD	Cement glands developed
PIT	Possibly infected with microsporidian parasite *Thelohania sp* - abdominal muscle opaque
DIT	Definitely infected with microsporidian parasite *Thelohania sp* - abdominal muscle white
BPA	Blue phase animal - blue colour
FWE	Female with eggs (DRC code) colour and approx# of eggs should be described with minimal handling of animal
FWY	Female with young (DRC code) colour and approx# of young should be described with minimal handling of animal

All crayfish are released except for three representatives of each species from each lake (maleFI, maleFII and female) to be kept as a reference collection. All atypical or unidentified animals should also be kept. Reserved animals are labelled and frozen and the information entered into the freezer log. Arrangements with Dr. David Barr at the Royal Ontario Museum have been made to contribute these animals to the ROM reference collection.

4. Night time vertical net tows

Night time vertical net tows are conducted to determine presence and abundance of diel migrating invertebrates. Gear types vary greatly for this type of sampling depending on species present and abundance.

Summary Recommendations for Core Data Collection

The wide spectrum of potential parameters and uncertainties about the degree of their variation both temporally and spatially have often led researchers to play it safe and collect 'no' data. This may not, in many cases, be as foolish as it seems given that the collection of bad data would be even more damaging and expensive. It is, however, possible to collect useful data with a minimum of effort.

Trophic Status - Trophic status information can be collected with one visit at spring turnover each year with only the expense of two TP tests. Three or four such visits will yield long-term trophic status data that can be used as inputs to models and to assess long-term changes in trophic status. Chlorophyll samples to support TP data are not required. Details on the collection of TP samples and assistance with the submission of samples can be obtained through the staff at the Dorset Research Center.

Transparency - Secchi depth determinations should be taken in any situation where monthly visits are planned. These will give annual ice-free averages which can be used to typify and compare between-lake transparencies and observe long-term trends. Care need only be taken not to omit the early and later portions of the season that might result from collecting data only during summer creels etc.

Oxygen - Oxygen profiles should be collected (minimally) circa Sept 1, +/- 2 weeks.

Zooplankton - Data bases would benefit from having at least one year of monthly zooplankton collections for each lake. These serve to typify the lake with respect to micro-crustaceans and may be useful as input to community or diversity ordination exercises. The use of such data provincially to compare lake processes cannot be assessed until the data is available and based on consistent collection by similar gear types. Also the data may eventually be used as background or pre-invasion data that can be used to assess the impacts of invading species.

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